Paroxysmal Nocturnal Hemoglobinuria

New ideas about an old disease

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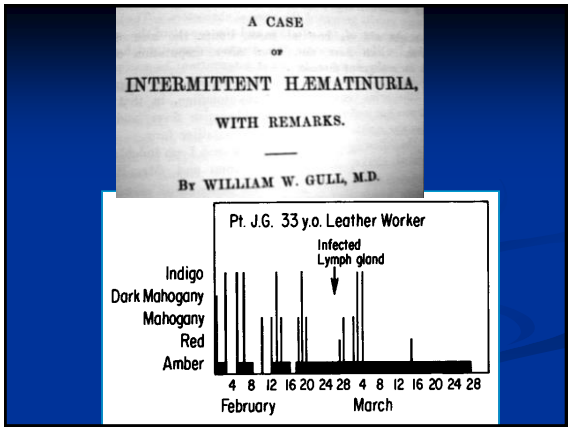
Objectives

- Try to answer some of the frequently asked questions about:
- The cause of the PNH
- The clinical presentation of PNH
- Diagnosing PNH
- The complications of PNH
- New treatments for PNH



What is PNH?

- A disorder of blood affecting all the cells which come the bone marrow.
- The disease is quite rare, only 10, 000 patients in the US and Europe.
- There is no ethnic preference for the disorder.
- It may present early or late in life.
- The manifestations may be "classic" or obscure.

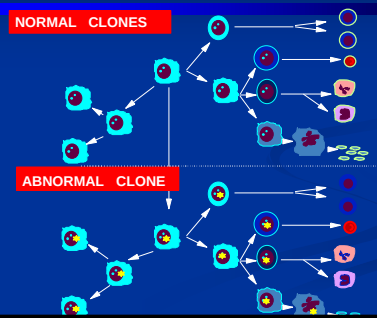
What is PNH?

- PNH is due to a mutation in a gene in a blood stem cell.
- The gene is called the **PIG-A** gene and is located on the X chromosome.
- In most cases of PNH, the change in the gene (mutation) is acquired, **not** something you are born with. When and why is unknown.
- The gene contains the genetic information for the **GPI anchors** which link proteins to the cell membrane

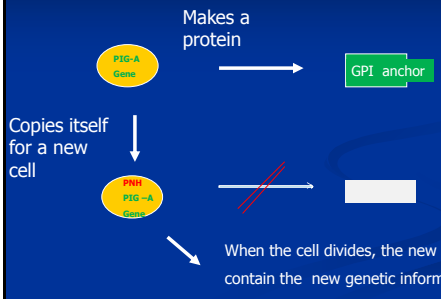
What is PNH?

- A mutation is a "mistake" or a "change" in the gene that arises during copying and is not corrected
- When the cell divides, the mutation is transmitted to daughter cells
- The effect of a mutation:
  - None
  - An altered protein (sickle hemoglobin)
  - No protein is produced as in PNH, hemophilia etc

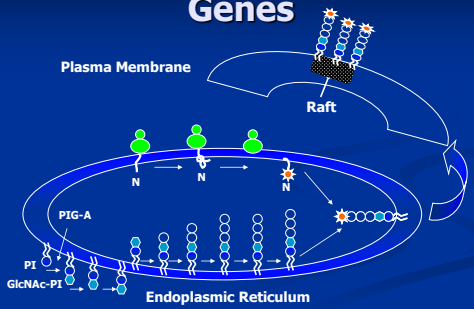
What is PNH?
Evolution of PNH in Marrow



What is PNH?



GPI-AP Biosynthesis: Involves 10 Steps and >20 Genes



What is PNH?

- As a result of the PIG-A mutation, there is little of no GPI anchor produced.
  - PNH II cells- mild reduction
  - PNH III cells- severely reduced.
  - When the anchor is reduced, certain proteins can't attach to the cells.
  - **The most important proteins for PNH are CD 59, CD55.**

What is PNH?

Many normal people have very small numbers (perhaps 6 per 1,000,000 bone marrow cells)

- **In PNH, the abnormal cells have an advantage and become a major population in the marrow and blood (anywhere from 1% to over 90%)**
  - **This may be a result of change in the immune system –inability to recognize something foreign.**
  - **Or it may be related an immune attack on and resultant damage to the bone marrow, leading to poor production of blood from the marrow**

PNH Clinical Features Aplastic Anemia

- **Some PNH patients have aplastic anemia or a history of aplastic anemia**
- **Many PNH patients have evidence of a bone marrow that doesn't work well or well enough to maintain normal blood counts**
- **Therefore, whatever causes aplastic anemia (immune suppression or dysregulation or damage to the stem cells) may allow PNH to develop**

What is PNH? Complement

- **Complement is a group of blood proteins that act together to help the body get rid of pathogens such as microbiological invaders**
  - **One of the ways it does this is by penetrating the membrane (outside surface) of the invading bacteria or viruses.**
  - **Complement induces inflammation and recruits inflammatory white blood cells to the area of injury or pathology. This can help trap and digest a pathogen or damaged cells**
  - **When complement proteins bind to PNH blood cells, the cells are destroyed.**

What is PNH?

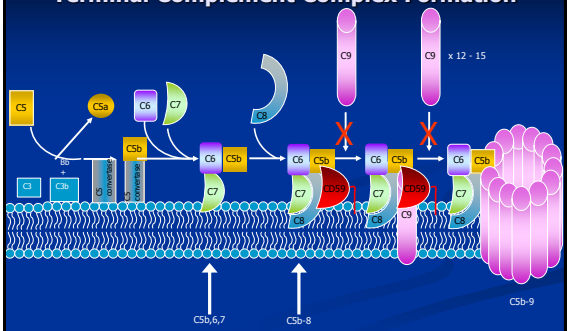
Complement circulates in an inactive form

- It is activated spontaneously and by a variety of events
  - **It is normally activated more at night**
  - **It is more active with infections, trauma, vaccinations, surgery, immune complexes, autoimmune diseases**

What is PNH? Complement

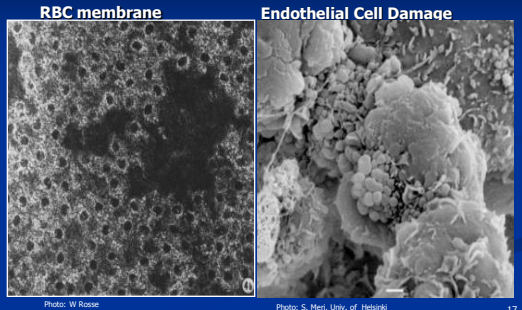
- Complement activity is regulated by proteins in the blood and on the membranes of the cell.
- **GPI linked proteins on the cell surface interfere with complement to prevent breakdown (lysis) of the cell membrane**
  - The most important of these are **CD59**, and **CD55** which is missing on the abnormal cells of PNH
  - For this reason, PNH red cells are extremely sensitive to very small amounts of activated complement

Absence of CD59 Allows Terminal Complement Complex Formation



Adapted from Cellular and Molecular Immunology AK Abbas, AH Litchman and JS Pober, 3rd Edition. 1991 WB Saunders; Philadelphia.

Multimeric C9 Lesions on PNH Cell membranes



Holers VM et al. *Immunol Rev.* 2008;223:300-316. Zipfel PF et al. *Curr Opin Nephrol Hypertens.* 2010;4:372-

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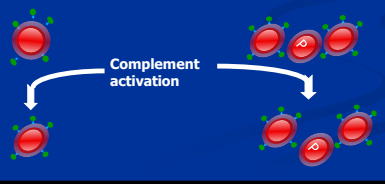
What is PNH?

- Complement attacks the red cells and they break up (hemolysis)
  - This releases hemoglobin (the red pigment in red cells) into the plasma
  - Causes anemia
  - Pieces of the membrane come off
- The white cells release granule contents and change to express other proteins
- The platelets form vesicles (membrane blisters) and activate

What is PNH?

Normal red blood cells are protected from complement attack by a shield of terminal complement inhibitors

Without this protective complement inhibitor shield, PNH red blood cells are destroyed



What is PNH? Clinical Features

- Some of the hemoglobin passes through the kidneys and into the urine, causing red to dark brown urine (hemoglobinuria)
  - This causes a loss of iron from the body
  - In the long run, this may damage the kidney
- Free hemoglobin binds nitric oxide causing vascular and smooth muscle spasm
- Causes inflammation

What is PNH Clinical aspects

- Vascular (arterial constriction, HBP)
- Pulmonary artery pressure increase (PHTN)
- Spasm of the esophagus
- Abdominal pain
- Erectile dysfunction
- Other symptoms such as "fatigue"
- Platelets are more "reactive"

What is PNH? Clinical Features

- WBC: **Granulocytes** - release content stimulating inflammation

  Monocytes - activate expressing TF which leads to blood clots. TF-Microvesicles
- Platelets become "activated". Receptor on the platelet for C5a

  They stick together and form clumps

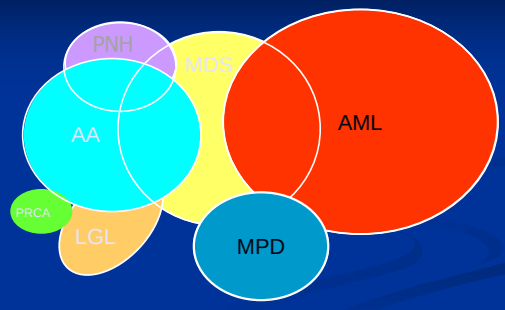
  The membrane changes, allowing them to bind to monocytes

  Pieces of the membrane come off (microvesicles)

What is PNH? Clinical Features

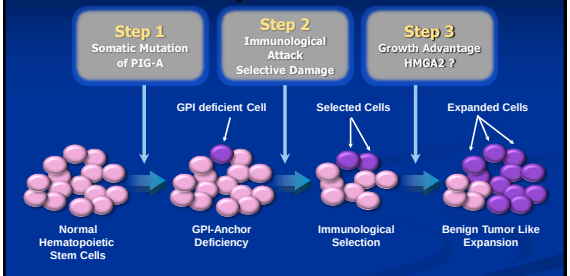
- Hemolytic anemia due to complement activation
  - Hemoglobinuria and kidney damage
  - Anemia to a variable degree
  - Effects of NO depletion- HBP, smooth muscle dystonia, reduced blood flow to the kidney and lungs
- Impaired bone marrow function

Bone Marrow Failure Syndromes



Young NS. *Ann Intern Med* 2002 Apr 2;136(7):534-46

Genesis of PNH : Clonal Expansion



- Expansion of the clone size is necessary to result in clinical PNH
- The need for both selection and expansion may explain the rarity of PNH

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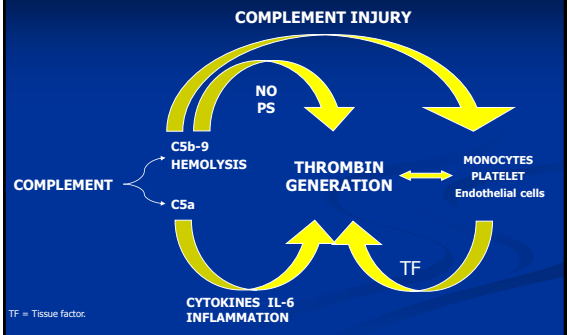
Adapted from Inoue N et al, Int J Hematol 2003;77 p107

What is PNH?
Clinical Features

- Thrombosis (Blood clots)
  - Often in unusual places (liver veins, abdominal veins, cerebral veins, dermal veins)
  - Can damage kidneys
- Fatigue – overwhelming, poor correlation to level of hemoglobin
  - inflammation
  - anemia
  - Pumonary Hypertension

Thrombosis in PNH Pathophysiology



TF = Tissue factor.

What is PNH
Diagnosis of PNH

- Historical test – Sucrose hemolysis, Hamm's test no longer used
- Flow cytometry on peripheral blood is the gold standard for diagnosing PNH[1]
- Both granulocytes and erythrocytes should be tested[2]
  - Erythrocytes alone are not sufficient due to hemolysis and dilution effect of transfusions
- Multiple monoclonal antibodies against GPI-anchored proteins (such as CD59 or CD55) are used[1,2]
- PNH blood cells (PNH clone) are cells that are missing GPI-anchored proteins

[1]Parker, et al. *Blood.* 2005;106:3699-3709.
[2]Hall & Rosse. *Blood.* 1996;87:5332-5340.

Management Options for PNH

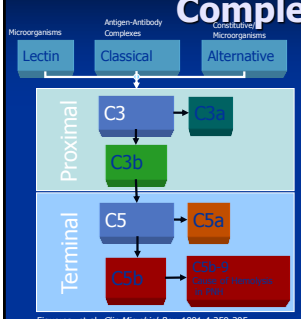
Generally conservative, supportive, and dependent on symptom severity[1,2]

- Transfusions
- Anticoagulants
- Supplements
  - Folic acid
  - Iron
  - Erythropoiesis stimulating agents
- Steroids/androgen hormones
- Allogeneic bone marrow transplant (limited eligibility)
- Complement inhibition

What is Soliris®?

- Monoclonal antibody (protein) that blocks complement at C5 preventing the formation of the terminal complement complex
- Quickly and markedly reduces hemolysis
  - Stops hemoglobinuria
  - Increases hemoglobin level
    - Reduces transfusions
    - Hemoglobin may not be quite normal

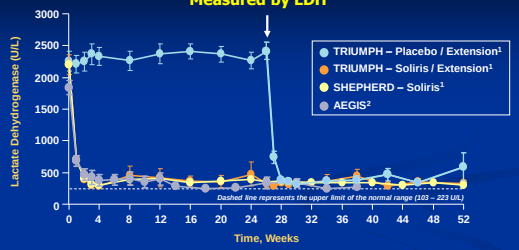
SOLIRIS Blocks Terminal Complement



- SOLIRIS binds with high affinity to C5
- Terminal complement activity is blocked
- Proximal functions of complement remain intact
  - Weak anaphylatoxin
  - Immune complex and apoptotic body clearance
  - Microbial opsonization

Figueroa, et al. *Clin Microbiol Rev.* 1991;4:359-395.
Walport. *N Engl J Med.* 2001;344:1058.
SOLIRIS™ (eculizumab) [package insert]. Alexion Pharmaceuticals; 2007.

Reduction in Chronic Complement-Mediated Hemolysis as Measured by LDH

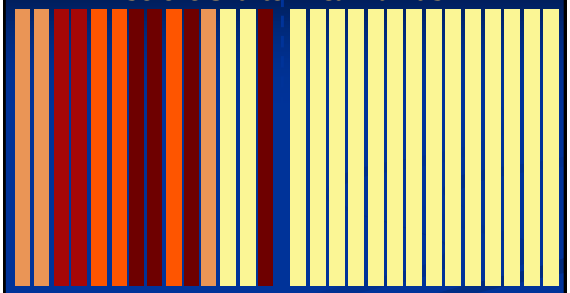


- TRIUMPH placebo patients switched to Soliris after Week 26
- All TRIUMPH patients entered the long-term extension study

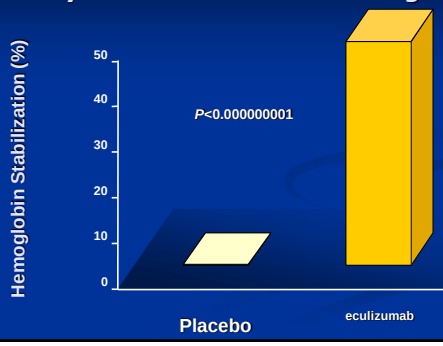
P<0.001 at all measured time points.

Please see full prescribing information for Soliris® (eculizumab). Soliris® (eculizumab) Summary of Product Characteristics. Alexion Europe SAS; 2012.
1. Hillmen P et al. *Blood.* 2007:110(12):4123-4128. 2. Kanakura Y et al. *Int J Hematol.* 2011;93:36-46.

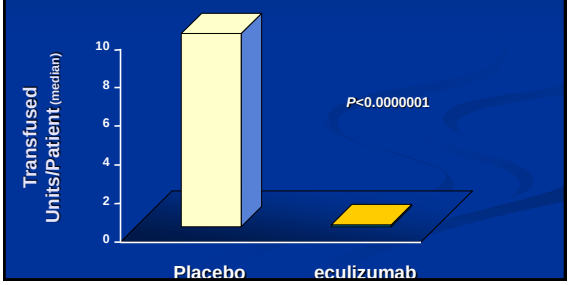
D.T., urine score 2 weeks before & after Eculizumab



Effect of Complement Inhibition on Ability to Maintain a Good Hemoglobin



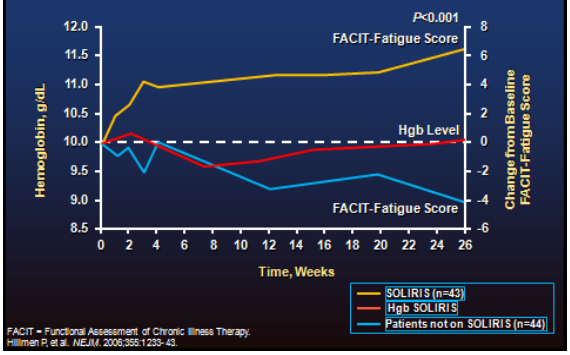
Effect of Soliris® on Transfusion in PNH



What is the effect of Complement inhibition in PNH

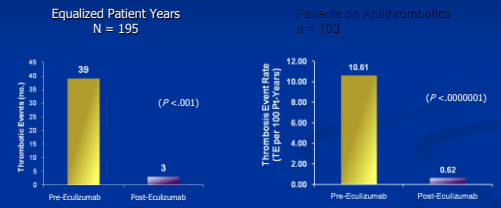
- Stops the symptoms associated with intravascular hemolysis
  - "Fatigue"
  - Esophageal and abdominal spasm
  - Erectile dysfunction
  - Improves sense of well being
  - Reduced the need for transfusion
- Appears to reduce thrombosis (blood clots)

TRIUMPH: Improvement in Fatigue (FACIT-F) Independent of Hemoglobin



FACIT = Functional Assessment of Chronic Illness Therapy.
Hillmen P, et al. *NEJM*. 2006;355:1233-43.

Effect of Eculizumab on the Thromboembolic Event Rate in PNH



92% reduction in TE events with eculizumab

94% reduction in TE event rate with eculizumab

Hillmen P, et al. *Blood*. 2007;110:4123-4128.

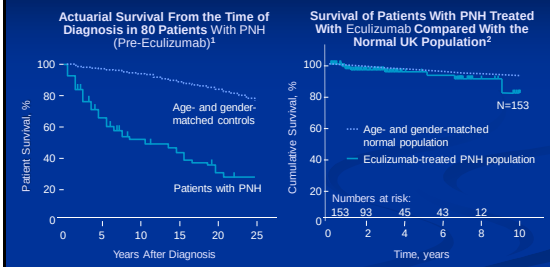
What is the Effect of Complement inhibition?

Improves kidney function
- reduced hemoglobinuria and iron deposition
- Reduced thrombosis

Improves hypertension

May in part be due to availability of nitric oxide

Long-term Clinical Benefits of Eculizumab in Patients With PNH



1. Hillmen P, et al. *N Engl J Med*. 1995;333(19):1253-1258. 2. Hill A, et al. *Blood*. 2012;120:Abstract 3472.

Pregnancy in PNH

Pre-eculizumab
- Increased fetal loss
- Increased risk of thrombosis*
- Increased transfusion requirements

Post Eculizumab*
- Improved fetal outcomes
- No major fetal abnormalities
- Reduced maternal morbidity
- Reduced risk of thrombosis*

Anticoagulation with heparin or LMWH required throughout the pregnancy and for 6-12 weeks post partum
**Eculizumab category C

Side Effects of EculizumabTreatment

- Susceptibility to sepsis by meningococcal organism
  - All patients must be vaccinated at least 2 weeks before starting Soliris
  - All patients must know to seek medical help at once when fever happens
  - All patients must carry cards describing this complication
- Headache – first week or 2
- Cost
- Inconvenience
  - Must be given every 12-14 days by vein

What Eculizumab Cannot Do

- Does not appear to improve impaired bone marrow function
  - Low white count or low platelet count may persist in some patients, especially if it is due to aplastic anemia
  - Other treatments may be indicated
    - Bone marrow transplantation
    - ATG and other immunosuppressives

When is Eculizumab Ineffective or Less Effective

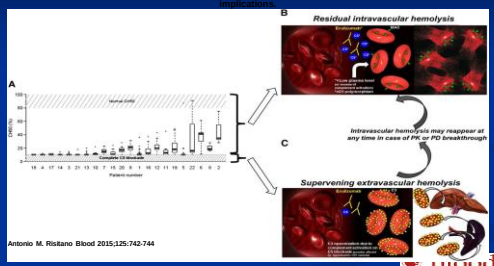
- Patient has been incorrectly diagnosed with PNH.
- C5 polymorphism
- Patient has a very small PNH clone (less than 10%)
- bone marrow failure- AA
- Breakthrough- inadequate dosing vs increased complement activation
- Extravascular hemolysis

Breakthrough Hemolysis with Eculizumab

- Eculizumab t1/2 10-12days
- Types of breakthrough – insufficient dose vs more rapid clearance vs increased complement activation
- day 8 LDH within normal limits but LDH increases prior to next dose on day 15
- CH 50 increases, measure a trough level trough (level decreases below 35 ug/ml) prior to day 15
- Recommended treatment:
- increase frequency of the dose to q 12 days (per PI) or give extra dose
- increase dose to 1200 mg q 14 days.
- Experimental- SQ daily vs 1210

Residual hemolysis in PNH:
Breakthrough vs extravascular hemolysis

Detection of residual complement activity in PNH patients on eculizumab, with their pathogenic and therapeutic implications.



Antonio M. Risitano Blood 2015;125:742-744

Extravascular clearance

- Accumulation of C3b/d/g on cells with clearance through the spleen.
- Complement Receptor (CR)1 modulates C3 deposition on RBC
- Genetic variants H/H, H/L, L/L
- polymorphism L/L(low expressor) increase C3 on membrane

7 times more likely to require transfusions

Treatment: transfusions, prednisone (?), splenectomy(?)
Experimental- APL2

Thank you